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IN-*VITRO* ANTI-ARTHRITIC ACTIVITY OF METHANOLIC EXTRACT OF *COUROUPITA GUIANENSIS* FLOWER

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Abstract:

The present study is aimed to evaluate the in-vitro anti-arthritic activity of methanolic extract of Couroupita guianensis flower using inhibition of protein denaturation model. Diclofenac sodium was used as a standard drug. Results revealed that the methanolic extract of Couroupita guianensis at different concentrations possessed significant anti-arthritic activity as compared to standard drug.

Key words: *Couroupita guianensis, anti-arthritic, protein denaturation.*

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INTRODUCTION:

Herbal medicine is unwritten science is well established in some cultures and tradition of developing countries. Arthritic conditions are treat ed using traditional medicine with considerable succe ss. Although various modern drugs are used to treat t hese type of disorders their prolonged usage may cau se severe side effects .So there is an urge to develop n ew therapeutic agents with minimum side effects [1]. Rheumatoid arthritis is highly inflammatory poly arth ritis, often leading to joint destruction, deformity and loss of function which has a worldwide distribution w ith an estimated prevalence of 1 to 2%. Prevalence in creases with age, approaching 5% in women over age 55. The average annual incidence in the United States is about 70 per 100,000. Both incidence and prevalen ce of rheumatoid arthritis are 2-3 times greater in wo men than in men [2].

The management of rheumatoid arthritis (RA) rests o n several principles such as drug treatment, which co mprises disease-modifying anti rheumatic drugs (DM ARDs) and also non-steroidal antiinflammatory drugs and gluco corticoids (GCs), as well as non pharmacological measures, such as physical, occupational and psychological therapeutic approaches, together may lead to therapeutic success[3].

Fig 1: *Couroupita guianensis* flower

MATERIALS AND METHODS:

Identification and collection of flower

The flowers of *Couroupita guianensis* were collected from the Mannargudi, Thiruvarur District, Tamil Nadu and India. They were identified and authenticated by Dr. John Britto, The Rapient Herbarium and Centre for Molecular Systematics, St.Joseph's college, Tiruchirappalli, Tamil Nadu, India.

Extraction and preparation of flower

The flowers were garbled and dried under shade and powdered. 25g of dried powdered flower materials were extracted separately with methanol using soxhlet apparatus for 48hrs.The solvent was distilled at lower temperature under reduced pressure and concentrated on water bath to get the crude extract which is stored in desiccator for future use.

In *vitro* anti arthritic activity

Protein Denaturation Method [4]

1. Test solution (0.5ml): It consist of 0.05ml of test solution of various concentrations (200-1000 µg/ml) and 0.45ml of Bovine serum albumin (5% aqueous soluti on)
2. Test control solution (0.5ml): It consist of 0.05ml of distilled water and 0.45ml of Bovine serum albumin (5% aqueous solution).
3. Product control (0.5ml): It consist of 0.05ml test solution of various concentrations (200-1000µg/ml) and 0.45ml of distilled water.
4. Standard solution (0.5ml): It consist of 0.05ml of Diclofenac sodium (200,400,600,800 and 1000µg/ml) and 0.45ml of Bovine serum albumin (5%aqueous solution).

PH was adjusted to 6.3 to all above solution by using 1N HCl. All the sample solution was incubated at 37 OC for 20minutes and the temperature was increased to 57 oC for 3 minutes. Allow the solution to cool for some time then add 2.5ml of Phosphate buffer to all above solution. The absorbance of the resulting solution is measured at 416 nm using UV visible spectrophotometer. The Percentage inhibition of protein denaturation was calculated as per the given formula

$$\text{Percentage Inhibition} = 100 - \frac{(\text{O.D. of test} - \text{O.D of product control}) \times 100}{\text{O.D. of control}}$$

Statistical analysis

Three replicates of each sample were used for each test to facilitate statistical analysis and the data were represented as mean ±standard deviation

RESULT AND DISCUSSION:

Table-1: Effect of *Couroupita guianensis* and Diclofenac sodium on inhibition of protein denaturation

s.no	Concentration(µg/ml)	Inhibition of protein denaturation by *Couroupita guianensis*	Inhibition of protein denaturation by Diclofenac sodium
1	200	33.3±1.55	29.1±1.33
2	400	58.3±1.58	42.3±1.36
3	600	75.8±1.63	69.9±1.55
4	800	79.1±1.68	72.8±1.52
5	1000	87.5±1.76	74.3±1.04

Each value represents means ± SD (n=3)

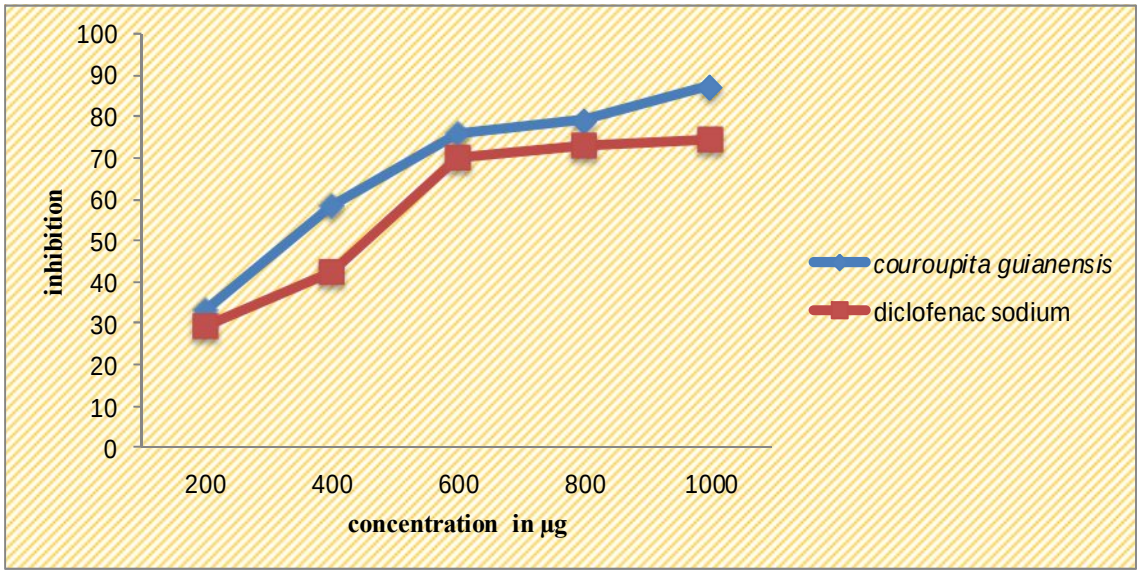

Fig.-2: Effect of *Couroupita guianensis* and diclofenac sodium on inhibition of protein denaturation

There are certain problems associated with animal use in experimental pharmacological research such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected or in vitro assessment of anti-arthritic property of methanolic flower extract of *Couroupita guianensis.*

The methanolic extract of *Couroupita guianensis* has showed significant activity at various concentrations and its effect was compared with the standard drug Diclofenac sodium. The maximum percentage inhibition of protein denaturation of *Couroupita guianensis* was observed as 87.5±1.76 at 1000µg/ml respectively as shown in Table 1. When compared to standard Diclofenac sodium was found out to be 74.3±1.04 respectively at a dose of 1000µg/ml. The production of auto antigen in certain arthritic disease may be due to denaturation of protein and membrane lysis. From the results (Figure 2) of our present study, it can be stated that methanolic extracts are capable of controlling the production of auto antigen and inhibits denaturation of protein and membrane lysis in rheumatic disease.

Denaturation of tissue proteins is one of the well documented causes of inflammatory and arthritic diseases. Production of auto-antigens in certain arthritic diseases may be due to denaturation of proteins in vivo [5, 6].Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding [7].

Literatures are now full of scientific documentation today regarding medicinal plants and they have potential to cure various human diseases [8]. Thus, this suberb future further encourage to manufacture a pharmaceutical products procured from medicinal plants as they are safe and dependable as compare to synthetic drugs, that are not only costly but also have adverse effects [9]. The naturally isolated anti-arthritic agents function by suppressing the different types of inflammatory mediators involved in inflammation process [10].

CONCLUSION:

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have various side effects, whereas plants still hold their own unique place, by the way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against arthritis and inflammation so as to exploit them as herbal anti-arthritic agents.

From the result of the present study, it can be stated that the methanolic extracts of *Couroupita guianensis* flower are capable of controlling the production of auto antigen and there by it inhibit the denaturation of proteins and its effect was compared with the standard drug Diclofenac sodium.

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